

Breeding profiles at the periparturient stage in captive red foxes (*Vulpes vulpes*) mating naturally or subjected to artificial insemination in Japan

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Abstract

Introduction: Breeding profiles at the periparturient stage in red foxes which mated naturally or were subjected to artificial insemination were retrospectively surveyed using 130 vixens during their reproductive seasons of 2012–2017 in Japan. **Material and Methods:** Natural mating vixens were encouraged a maximum of three times with the same male, while artificial insemination was conducted using frozen-thawed semen with the bovine semen extender as a diluent. **Results:** With natural mating, conception rates after one, two, and three copulations were 55.8%, 68.0%, and 85.7%, respectively, showing a significant difference between the rates for one and three copulations. Conception rates with artificial insemination were 82.4%. Mean gestation periods were between 52.1 and 53.3 days in all groups. Mean litter sizes were 3.7–4.3 cubs with natural mating, and 4.4 cubs with artificial insemination. Although some sporadic and inconsistent changes in litter sizes were noted between primiparous and multiparous groups, these were of doubtful clinical importance. **Conclusion:** This is the first report from Japan concerning basic breeding events of red fox vixens in captivity.

Keywords: red fox, natural mating, artificial insemination, breeding profile.

Introduction

Although foxes have been defined as economically valuable fur-bearing animals (4, 5, 7, 8), in Japan they are now raised only in zoos. In maintaining a small herd, cumulative knowledge regarding vaginal mating (natural mating) or artificial insemination is essential to avoid genetic degeneration and to maximise fertility for breeding. In previous research, it was suggested that a single peak magnitude (over 350 units = Ω) of vaginal electrical resistance (VER) in vixens prior to coupling together with measurement of insertion time of the penis in the vagina during mating (coital-lock time) may be an

expedient predictor of conception immediately after mating (13). In further work, the optimum conditions for semen collection in electroejaculation were investigated with frozen semen using the bovine semen extender as a diluent for artificial insemination (12). In the present study, breeding profiles such as conception rate, gestation period, and litter size in red foxes mating naturally or subjected to artificial insemination were retrospectively surveyed using 130 vixens in captivity, because there was scant basic information at the periparturient stage in Japan. Additionally, to clarify whether there is a difference in breeding profiles between primiparous and multiparous vixens, we analysed them statistically.

Material and Methods

Animals. Healthy red foxes (*Vulpes vulpes*) maintained at the Zao Fox Village (latitude: 38°02'14"; longitude: 140°32'02"; altitude 700 m, Miyagi, Japan) were used in the study. In the Fox Village, about 400 foxes including approximately 60–70 males and 30–40 females, which are kept in an outdoor enclosure (0.5 ha), have been raised since its opening as a petting zoo in 1985. Age and body weight ranges of foxes used were 2–5 years in both sexes and 8–11 kg in females and 9–11 kg in males. The foxes used were considered “healthy” based on their appetites, conditions of faeces and urine, fur appearance, and vital signs (rectal temperature, pulse, and respiratory rate). They were given feed once daily in the morning comprising 400–500 g of a mixture containing dog food and fish, horse, and beef meals (total energy: approximately 150 kcal/100 g) and tap water *ad libitum*. Rations of mixed-feed mash (a protein level of 15%, fat 5%, and carbohydrate 3%) were given to both males and females throughout the experiment, although their amounts in females increased from 400 g to 500 g during the lactation period. Each fox was identified individually with a microchip (11 mm × 2.2 mm, Trovan, UK). All foxes were given ivermectin (6% Ivermec Powder, Fujita Pharm, Japan) four times between April and November and praziquantel (Drontal Plus, Bayer, Japan) once between May and June mixed with the diet for prophylaxis of heartworm and *Echinococcus* infections. Males and females were kept in separate enclosures during the non-reproductive season (April to December) and data on vixens during their reproductive season (January to March) were analysed.

VER measurement. VER measurement in vixens has become a routine technique in modern fox reproduction. It has been recognised as reaching a maximum around ovulation and therefore has been used for detecting the optimum mating time of farmed vixens (1, 5, 8). In the reproductive season starting in January, 2–3 stud males were introduced to 8–10 females for approximately two months under video camera monitoring. When males began to chase after certain females, the oestrus of these vixens was assessed by determining VER after confirming visually that the vulva had become well swollen. The VER was measured twice at 2 min intervals by an ovulation detector (Dog Ovulation Detector; Draminski, Poland) attached to a vaginal probe. The probe rod was inserted about 7–10 cm up to the cervix for 30 s until the value became stable. The lower VER value at 2 min intervals was adopted. Immediately after natural mating or artificial insemination, vixens were placed in a hand-made modified nursery box (90 cm × 50 cm × 40 cm), which was divided into three dark sections and combined with a wire breeding cage (90 cm × 50 cm × 60 cm) with an entrance tunnel, for the duration of their pre-parturition and nursing stages totalling 80 days.

Further details of the nursery box and breeding cage formations for this experiment were described in other reports (2, 9–11). All foxes were fasted for at least 20 h before the mating.

Natural mating. The study was carried out using 96 vixens during 2012–2017. The initial mating was arranged on the first day after a single peak of VER was higher than 300 units. Couplings of vixens showing aggressive or avoidance behaviour towards males were immediately abandoned. Vixens which accepted males were monitored with the video camera. The coupling having been to fruition or not was judged by the presence of spermatozoa in the vaginal smears. If spermatozoa were not confirmed microscopically, the vixen was housed together with the same male again after confirming that her VER was higher than 300 units. Coupling of vixens was encouraged a maximum of three times with the same male. Numbers of vixens which copulated once, twice and three times were 43, 25, and 28, respectively. Forty-five male animals were nominated as stud fox candidates, taking into consideration bloodline records to avoid coupling between close relatives. Most of the males used were imported as original stud foxes from Norway, and their male offspring were also used in this work. Using semen obtained in advance by electroejaculation (12), only individuals showing high viability with motility and suitable numbers ($250\text{--}600 \times 10^6$ cells/mL) of spermatozoa were selected as stud foxes. After screening, 11 males were eligible and were used as studs five to eight times each for six years.

Artificial insemination. The investigation was undertaken using 34 vixens during 2015–2017. Artificial insemination was conducted by use of frozen-thawed semen twice on the first and second days after a single peak of VER over 350 units, as with natural mating. Eleven males were used as semen donors. The procedure for artificial insemination with the preparation of frozen-thawed semen using the bovine semen extender has been already reported (12). The bovine semen extender was provided from the Miyagi Prefectural Livestock Experiment Station (Miyagi, Japan). There was no large difference in components between the bovine and fox extenders (5), although a minor difference in their concentrations was noted.

Conception rate, gestation period, and litter size. Conception rates (number of pregnancies/number of vixens used) were calculated and represented as percentages. Vixens that delivered were divided into primiparous and multiparous (2–4 gestations) groups. The gestation period was calculated from the last date of coupling (if the vixen mated naturally) or from the date of second procedure (if artificially inseminated) to the parturition date, which was determined by neonatal pup call *via* the video monitoring. The litter size was confirmed 4 weeks later (week 4), just after weaning, because vixens after birth were extremely nervous.

Table 1. Breeding profiles in vixens subjected to natural mating or artificial insemination

Instances of copulation	Natural mating ^{a)}				Artificial insemination
	One	Two	Three	Total	
No. of vixens used	43	25	28	96	34
Conception rate ^{b)} (%)	55.8	68.0	85.7*	67.7	82.4
Gestation period (days)	53.3 ± 0.2 ^{c)}	52.1 ± 0.3	52.4 ± 0.3	52.6 ± 0.2	53.1 ± 0.2
Litter size (cubs) in week 4	3.7 ± 0.2	4.2 ± 0.3	4.3 ± 0.2	4.1 ± 0.1	4.4 ± 0.2

^{a)}Coupling was attempted a maximum of three times with the same vixen and male. ^{b)}No. of pregnancies/no. of vixens used.

^{c)}Values represent the mean ± standard error of the mean (SEM) *P < 0.05 vs. the initial challenge in the natural mating group (Chi-squared test)

Statistical analysis. Numerical data are expressed as the mean ± standard error of the mean (SEM). Conception rates were compared using Fisher's exact test or the chi-squared test. For comparing two groups, each continuous variable was first tested for homogeneity of variance by *F*-test. If the variance in the case of normal distribution was homogeneous, intergroup differences were assessed by Student's *T*-test. Otherwise, intergroup differences were analysed by Welch's *T*-test. For comparison of gestation period and litter size among the cases of one, two, and three copulations, the Steel-Dwass test was applied. A probability level of $P < 0.05$ indicated statistical significance.

Results

Breeding profiles in vixens which mated naturally or were subjected to artificial insemination are shown in Table 1. With natural mating, conception rates in cases of one, two, and three copulations were 55.8% (24/43 vixens), 68.0% (17/25 vixens), and 85.7% (24/28 vixens), respectively, showing a significant difference between one and three copulations. The overall conception rate with natural mating was 67.7% (65/96 vixens), and the rate with artificial insemination was 82.4% (28/34 vixens). Mean gestation periods were between 52.1 and 53.3 days (reference range 52–56 days) in all groups. Mean litter sizes in week 4 were 3.7–4.3 cubs (3–5 cubs) from natural mating, and 4.4 cubs from artificial insemination.

In vixens ($n = 65$) pregnant by natural mating at all instances of copulation, no significant difference was noted in conception rates, gestation periods, or litter sizes between primiparous ($n = 30$) and multiparous ($n = 35$) vixens (data not shown). As depicted in Fig. 1, statistically significant differences in litter sizes were observed for conceptions from natural mating when there was one (12 primiparous vs. 12 multiparous vixens), two (5 primiparous vs. 12 multiparous vixens), and three (13 primiparous vs. 11 multiparous vixens) copulations, and between litter sizes for conceptions by artificial insemination (12 primiparous vs. 16 multiparous vixens).

Discussion

Using 130 red fox vixens in captivity, breeding profiles including conception rate, gestation period, and litter size in animals which mated naturally or were subjected to artificial insemination were retrospectively surveyed in Japan.

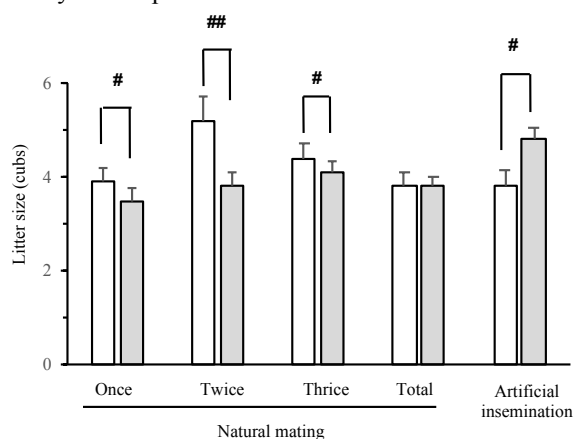


Fig. 1. Comparison of litter sizes between primiparous (open bars) and multiparous (grey bars) groups in cases of one, two, and three copulations (natural mating), and between them with artificial insemination in vixens. #P < 0.05 and ##P < 0.01 vs. the primiparous group (Student's *t*-test or Welch's *t*-test)

Conception rates with natural mating seemed to be mating-instance dependent with rates increasing with repeated instances (Table 1). Low conception rates from a single copulation may be due to immature sexual susceptibility in vixens at the initial event. Although the total sum (67.7%) of conception rates from natural mating tended to be lower than conception rates (82.4%) from artificial insemination, there was no statistical difference between them. Conception rates from artificial insemination obtained under the conditions of this study were nearly consistent with previous cumulative data (1, 4, 5) in red foxes or silver foxes (which are a colour mutation of red foxes). However, there were large differences in breeding and management conditions between the present and previous experiment. Meanwhile, males that had successfully bred in the past were likely to exhibit a high conception rate. Further studies will need to

support our findings with increased sample numbers of vixens and stud males.

Neither abortion nor dystocia was video-recorded in any of the groups throughout the trial. Mean gestation periods were between 52.1 and 53.4 days in the natural mating and artificial insemination groups, which were consistent with the previous reports (1, 4, 7). Mean litter size was 3.7–4.3 cubs with natural mating, and 4.4 cubs with artificial insemination, showing a subtle difference. No cub deaths were observed during the weaning periods of up to 80 days on the screen recordings. According to a previous report on red foxes (3), the average litter size varied from 1.3 to 3.5 cubs, but mean placental scars were indicative of 5.2–11.2 conceptions per litter. These implied that high rates of embryonic, foetal, and cub losses may occur during the perinatal period. Similarly, in blue foxes (which are a colour mutation of arctic foxes), it was reported that the mean litter size was 10.8 cubs at birth, but this declined to 7.8 cubs at weaning, while approximately 2% of the cubs were stillborn and 80% of cub deaths occurred during the first week of life (11).

Social suppression of reproduction in breeding cages was a widely recognised phenomenon in foxes (2, 9–11). For example, maternal behaviour related to survival of cubs was reported to be affected by the type of space provided, these possibly being nursery cages such as traditional cages (with only one room and a wide entrance) and modified boxes (with a narrow entrance tunnel, and kept in darkness). When pre- and post-parturition maternal behaviour of silver fox vixens was compared between these two accommodation types, the vixens in the modified box spent more time sleeping inside the box, cleaning newborn cubs, and grooming cubs while lying down, and less time looking out of the box (2). Consequently, cub mortality was reported to be lower in the modified box than in the traditional cage. In the present investigation, although the ratios of embryonic loss were not clear, management and housing conditions in the modified nursery box with a tunnel were thought to be well maintained, evidenced by the lack of abortion or cub losses.

Although some sporadic and inconsistent changes in litter sizes were seen between primiparous and multiparous groups in cases of natural mating or artificial insemination (Fig. 1), these were considered minor changes, within the reference ranges, and of doubtful clinical importance because of the small sample size.

In conclusion, this is the first report from Japan concerning basic breeding events at the periparturient stage of red fox vixens in captivity.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: All procedures were carried out in accordance with the animal welfare rules of the Zao Fox Village based on the Guidelines for Animal Experimentation (6), preceded by the approval of the Ethics Committee.

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